Amendments to the Drawing

Replacement drawing sheets are attached to this Response as Appendix A. These replacement sheets are identical to the original drawing sheets comprising Figures 1-12 submitted with the original application and have been amended to include the appropriate labels and to improve legibility. Accordingly, Applicants respectfully request that all objections to the drawings be withdrawn.

Remarks/Arguments

Amendments to the Claims

Claims 1 and 9 have been amended to recite "limiting the contacting time for a given volume of the mixture in contact with the solid phase at any one time". Support for this amendment is found on page 7, lines 1-4.

Support for new claims 10-14 is found on page 12, lines 7-27.

Drawings

Formal drawings that comply with 37 C.F.R. §1.84 and §1.121 are attached.

Priority

The Examiner asserts that the present application is not in condition to claim priority to the previously filed application number 08/277,225, now United States Patent No. 6,664,114, because the claims drawn to an assay in which the contacting time between a solid phase reactant and a sample is limited to less than about 1 second, 0.48 seconds, 0.12 seconds or 0.06 seconds are not supported in the parent application. Applicant respectfully submits, as discussed in more detail below, that the present application is a continuation of application number 08/277,225, now United States Patent No. 6,664,114, not a continuation-in-part as suggested by the Examiner. The two applications contain identical subject matter. Below, Applicant specifically points to support for the recited times in the specification. The priority claim is proper and this objection should be withdrawn.

Specification

The Examiner has objected to the specification, asserting the status of the parent application needs to be updated. The first paragraph of the specification has been amended to indicate that the parent application no. 08.277,225 has issued as U.S. Patent No. 6,664,114.

The Examiner has also objected to the specification, asserting that the specification fails to provide proper antecedent basis for the contact times recited in claims 1, 8 and 9. Claim 1 has been amended to recite contacting times "for a given volume of the mixture in contact with the solid phase at any one time being selected from the group consisting of a time limited to less than about 1 second, 0.48 seconds, 0.12 seconds, and 0.08 seconds". Applicant submits that the

specification does in fact provide proper antecedent basis for the contacting times recited in claims 1, 8 and 9, as currently amended.

The present claims recite a method of measuring the amount of analyte in a solution. The specification discloses that an important element of the invention is that the contact time of the analyte and reagent containing solutions with the solid phase be relatively short (page 7, lines 1-2). The specification also explicitly provides that in a preferred embodiment, "only a relatively small volume of the analyte (about 2 microliters) is in contact with the solid phase material at any given time" (page 7, lines 2-4) (emphasis added). Regardless of the flow rate of the mixture over the solid phase, the amount of mixture in contact with the solid phase at any given time is a constant, and depends solely on the volume of interstitial space present within and immediately surrounding the solid phase. Thus, in the preferred embodiment referred to above, there is about two microliters of interstitial volume present within and immediately surrounding the solid phase. It is elementary that the faster the flow rate of the mixture over the solid phase, the less time a given volume (e.g., two microliters) of the mixture is in contact with the solid phase.

Example 3 (page 24, line 8 through page 28, line 5) describes a series of competition-like experiments in which the flow rate over the solid phase was varied. Table 1 (page 25) shows the results of these competition-like experiments where the flow rate was 250, 750 and 1500 microliters per minute. The contacting time of 0.08 seconds recited in claim 1 can be simply calculated using a 2 microliter interstitial volume and a flow rate of 1500 microliters per minute as follows:

1 minute/1500 microliters X 60 seconds /1 minute X 2 microliters = .08 seconds.

The contacting times of 0.12 and 0.48 seconds recited in claim 1 can be similarly calculated using flow rates of 750 and 250 microliters per minute, respectively. One of ordinary skill in the art would understand that contacting times for a given volume of the mixture in contact with the solid phase at any one time will vary inversely with the flow rate of the mixture over the solid phase and would be able to easily calculate the recited contacting times from the experimental data of Example 3.

Support for the contacting time of 1 second recited in claim 1 is found on page 16, lines 27-30 ("the contacting time... will be less than about one minute, preferably less than about ten seconds and most preferably *less than about 1 second*" (emphasis added)).

Applicant thus requests that the objection to the specification for failing to provide proper antecedent basis for the claimed contacting times be withdrawn.

Rejections under 35 U.S.C. §112

Applicant submits that the present amendments to the claims and arguments render the Examiner's rejections under the second paragraph of 35 U.S.C. §112 moot. Specifically:

The Examiner has rejected claim 1 for failure to provide antecedent basis for the recited mixture in step (d). Claim 1, part (d) has been amended to recite "the mixture produced in step (b)" to provide a proper antecedent basis.

The Examiner has rejected claim 5, asserting that it is unclear how the step of combining a sample with a regent influences the concentration of the reagent. Claim 5 has been amended to recite "wherein the step of mixing comprises adding said second ligand to the sample such that the second ligand and the analyte are present at approximately similar concentrations" to more clearly specify that the concentration of the second ligand in the mixture does depend on how much second ligand is added, as stated by the Examiner.

The Examiner has rejected claim 6, asserting that it is confusing because it is unclear if the recited solid phase is the same one recited in part (c) of claim 1 or if it is a different solid phase. Claim 6 has been amended to recite "the solid phase" to indicate that it refers to the same solid phase provided in step (c) of claim 1.

The Examiner has rejected claims 1, 8 and 9 under 35 U.S.C. §112 second paragraph, asserting that the phrase "contacting time for a column volume of mixture" is unclear. Applicant has amended these claims to recite a "contacting time for a given volume of the mixture in contact with the solid phase at any one time" in order to more clearly specify that the recited contact times are in reference to the flow rate of the mixture over the solid phase. As the mixture flows continuously over the solid phase, the volume of mixture in contact with the solid phase at any one time is a constant and depends solely on the volume of interstitial space present within and immediately surrounding the solid phase. The faster the flow rate of the mixture over the solid phase, the less time a given volume of the mixture is in contact with the solid phase. One of ordinary skill in the art would understand that "a contacting time for a given volume of the mixture in contact with the solid phase at any one time" will vary inversely with the flow rate of the mixture over the solid phase.

The Examiner has rejected claim 7 under 35 U.S.C. §112 second paragraph, asserting that it appears to recite that in the absence of analyte, less first ligand/second ligand complexes are formed. Applicant respectfully submits that the Examiner has misread claim 7. The Examiner is correct in stating that the analyte and the second ligand compete for binding with the first ligand. However, claim 7 recites mixing a sample containing an analyte with the second ligand such that the "amount of unbound second ligand in the mixture is thereby reduced". Claim 7 further recites contacting this mixture with the first ligand bound to a solid phase. Any second ligand that was not bound by the analyte in the previous step is available to bind to the first ligand and form first ligand/second ligand complexes. The number of first ligand/second ligand complexes is thus "smaller than it would have been had said analyte/second ligand complexes not been formed", as recited in claim 7. In contrast to the Examiner's reading of claim 7 to recite the addition of no analyte, claim 7 clearly specifies that analyte in a sample is mixed with second ligand and that the number of first ligand/second ligand is thereby reduced when the mixture containing analyte/second ligand complexes is contacted with the first ligand.

Rejections under 35 U.S.C. §103

The Examiner has rejected claims 1 and 4-9 under 35 U.S.C. §103 as being unpatentable over Pollema et al. (Anal. Chem., 64:1356-1361, 1992) in view of Friguet et al. (J. Immun. Meth., 77:305-319, 1985) and Woods et al. (U.S. Patent No. 4,469,787). Applicant traverses this rejection and requests its withdrawal for the following reasons:

Traditional competition assays are performed by adding a known amount of labeled analyte analog or analyte conjugate to a sample containing an unknown quantity of unlabeled analyte. The mixture is then incubated with a ligand that binds either the analyte or the analyte conjugate. In traditional competition assays, the binding is allowed to reach equilibrium conditions.

In contrast, the present claims recite a method of detecting and measuring the amount of analyte in a sample by that improves on the methodology of traditional competition and sandwich assays. According to the method recited by the present claims, a sample with an unknown quantity of analyte is incubated with a second ligand such that analyte/second ligand complexes are initially formed. These complexes are then contacted with a solid phase containing a first ligand that is able to bind second ligand that is not complexed with the analyte. The present claims recite contacting a given volume of a mixture containing the preformed

analyte/second ligand complexes with the immobilized first ligand for less than about 1 second, 0.48 seconds, 0.12 seconds, and 0.08 seconds. Such limited contact times ensure that substantially no dissociation of the preformed analyte/second ligand complex occurs. Thus, only excess second ligand that failed to bind the analyte in the first step is available to bind the first ligand immobilized on the solid phase.

Pollema discloses a method to measure antibody binding kinetics that employs a sequential injection strategy and utilizes magnetic beads coated with antibodies. The antibody-coated magnetic beads are held in place magnetically to form an immobilized reaction surface. A sample mixture containing labeled protein is then aspirated into the reaction coil, the flow is stopped and the labeled protein is incubated with the immobilized magnetic beads for a period of time. The sample mixture is then discharged from the reaction coil and the amount of labeled protein not bound to the magnetic beads is measured. By measuring the amount of labeled protein that did not bind the antibody-coated beads, Pollema is able to calculate the amount of protein that bound the magnetic beads during the incubation period. By calculating the amount of protein that binds the antibody-coated beads during various incubation times, Pollema is thus able to determine the kinetics of antibody binding.

Pollema also presents the initial results of a competitive immunoassay using this approach. By "spiking" a sample containing an unknown amount of unlabeled antibody with a known amount of labeled antibody and introducing the spiked sample to the immobilized immunomagnetic beads, Pollema is able to determine the amount of unlabeled antibody in the sample. If the sample contains little unlabeled antibody, the labeled antibody will undergo maximal binding and only a small amount will be detected when the sample is discharged from the reaction coil. Conversely, if the sample contains a large amount of antibody, the unlabeled antibody will occupy most of the available sites on the beads and a large amount of labeled antibody will be detected when the sample is discharged from the reaction coil. This is nothing more than a standard competition assay in which the solid phase is created via magnetically held antibody-coated beads. Pollema fails to teach or suggest limited contact times such that true competition between the labeled antibody, unlabeled antibody and the immunomagnetic beads does not occur. In fact, in comparing the results of his initial competition assay to traditional competition assays, he states, "in its current state, there is no advantage in sensitivity to the proposed method" (page 1357, column 1, lines 1-2 of the text). In contrast, the present claims recite a method that achieves a substantial increase in sensitivity over standard competition

assays by limiting the contact time between the reaction components such that substantially no dissociation of existing analyte/second ligand complexes occurs.

Not only does Pollema fail to teach or suggest such limited contact times, he also fails to show any appreciation for, or motivation for utilizing, an assaying system where pre-formed analyte/second ligand complexes do not dissociate while in contact with the immobilized first ligand, as recited in the present claims. Indeed, Pollema states that his competition assay is optimized if there is a slight excess of labeled antibody compared to immobilized first ligand (page 1359, column 2, lines 15-17 of the text). Using Pollema's method, the presence of excess first ligand skews the results because as the analyte/second ligand complexes dissociate, all of the dissociated second ligand reacts with the first ligand on the solid phase. By limiting contact times such that no significant amount of analyte/second ligand complex dissociation occurs, the presently claimed invention avoids this problem. Indeed, unlike Pollema, the presently claimed methods can be performed under conditions of substantial first ligand excess. *See, e.g.,* claim 6.

Neither Friguet nor Woods cures the deficiencies of Pollema. Friguet teaches a method of determining affinity constants in solution by using an indirect ELISA. According to the teaching of Friguet, a labeled antibody is incubated in solution with an antigen until equilibrium is reached (analogous to formation of the analyte/second ligand, as recited in the present claims). The solution is then subjected to a standard ELISA and the amount of labeled antibody that is not bound to the antigen is measured (analogous to contacting the mixture with immobilized first ligand, as recited in the present claims). However, Friguet teaches that the contacting times between the analyte/second ligand complex and the immobilized first ligand are either 15 minutes or one hour (see Materials and Methods, page 309, lines 9-11 and lines 19-21), much longer than the contact times of 1 second, 0.48 seconds, 0.12 seconds and 0.08 seconds recited in the present claims.

Furthermore, the method taught by Friguet only works when the amount of unbound antibody (corresponding to the second ligand of the present claims) retained by the first ligand in their ELISA is only a small fraction of the total free antibody (see page 314, first full paragraph). Due to the long incubation times taught by Friguet, this can only be accomplished when the amount of first ligand is limited in comparison to the amount of the labeled antibody. In contrast, the method recited in the present claims can be performed under conditions where the immobilized first ligand is present at substantial excess over the unbound second ligand. *See, e.g.,* claim 6. Thus even at the very short contact times recited in the present claims, it is

possible for a substantial portion of the unbound labeled second ligand to be retained by the immobilized first ligand.

The Examiner cites Woods for teaching a method for quantitatively determining the presence of a ligand in a sample in a *sandwich assay*. Traditional sandwich assays are performed by binding the analyte to a first ligand immobilized on a solid phase. A labeled second ligand that also binds the analyte is then added, forming a first ligand/analyte/second ligand complex. By measuring the amount of labeled second ligand bound to the solid phase, the amount of analyte in solution can be determined. Traditional sandwich assays suffer from the common problem of non-specific binding of the second ligand to the solid phase, independent of the analyte. Thus, traditional sandwich assays often indicate a higher level of analyte than actually exists. The method recited in claims 8 and 9 takes advantage of the fact that non-specific binding of the second ligand to the solid phase is generally slower than specific binding of the second ligand to the analyte. By limiting the contact time that the second ligand is in contact with the first ligand/analyte complex, non-specific binding is preferentially reduced in comparison to specific binding.

Woods teaches an improved sandwich assay in which the previously required step of enzymatically removing the F_c portion of the antibodies used in the sandwich assay is obviated. Woods provides no teaching or suggestion relevant to the present *competition-like assay* claims (claims 1-7). With respect to the sandwich assay claims (claims 8 and 9), Woods does not provide any teaching or suggestion, nor does he show any appreciation for, limiting the contact time between the labeled second ligand and the solid phase is limited such that non-specific binding is minimized, as recited in claims 8 and 9.

Contrary to the Examiner's assertion, it would not have been obvious at the time the invention was made to use the method taught by Pollema to either a competitive or sandwich assay as taught by Friguet and Woods. None of these references contains a teaching or suggestion that the contact times between the components of a competition or sandwich assay be limited to the times recited in the present claims, such that competition for binding between the components does not occur. Thus, even if the teachings of these references were combined, such a combination would not result in the method recited in the present claims which recite improved methods of detecting the presence or quantity of an analyte in solution by limiting contact times between the reaction components.

The Examiner has also rejected claims 2 and 3 under 35 U.S.C. §103 as being unpatentable over Pollema et al. in view of Friguet et al. and Woods et al. and further in view of Freytag et al. (Clin. Chem., 30(9):1494-1498, 1984). Applicant traverses this rejection and requests its withdrawal for the following reasons:

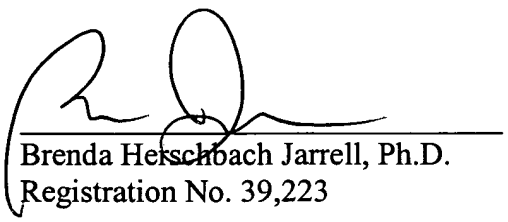
Freytag teaches an affinity-column immunoassay in which he demonstrates that an analog of an analyte bound to the solid phase, as opposed to the actual analyte, results in a higher assay sensitivity.

All of the arguments pertaining to Pollema, Friguet and Woods above are restated and incorporated here. Furthermore, Freytag does not cure the deficiencies of these references since Freytag does not teach or suggest limiting the contact time between the analyte/second ligand complex and the immobilized first ligand to the short times recited in the present claims, such that competition for binding between the components does not occur. Thus, contrary to the Examiner's assertion, combining the assay of Pollema as modified by Friguet and Woods to the method taught by Freytag would not result in the method recited in the present claims, which specifically recite limited contact times in order to substantially eliminate competition between the first ligand and the analyte/second ligand complex.

In light of these Remarks and Amendments, Applicant respectfully submits that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

Please charge any applicable fees associated with this filing, or apply any credits, to our Deposit Account No. 03-1721.

Respectfully Submitted,



Brenda Herschbach Jarrell, Ph.D.
Registration No. 39,223

Patent Department
Choate, Hall & Stewart
Exchange Place
53 State Street